PB81-129512

Succession of Microfungi in Estuarine Microcosms Perturbed by Carbaryl Methyl Parathion and Pentachlorophenol

(U.S.) Environmental Research Lab.
Gulf Breeze, FL

1980

U.S. Department of Commerce
National Technical Information Service
NTIS.

Botanica Marina
Vol. XXIII, pp. 129–131, 1980

Succession of Microfungi in Estuarine Microcosms Perturbed by Carbaryl, Methyl Parathion and Pentachlorophenol*

W. L. Cook, Denise Fiedler** and A. W. Bourquin**

Department of Biology, Georgia State University, Atlanta, Georgia 30303
and
***U.S. Environmental Protection Agency Environmental Research Laboratory, Gulf Breeze, Florida 32561*

(Received October 10, 1979)

Abstract

The effects of carbaryl, methylparathion and pentachlorophenol on the microfungal succession of an estuarine microcosm were examined. Resident fungi were succeeded by *Fusarium* in the carbaryl-treated microcosm; by a fungus in the *Penicillium chrysogenum* series in the methyl parathion-treated microcosm; and by a fungus in the *Penicillium canescens* series in the pentachlorophenol treated microcosm. Small quantities of $^{14}CO_2$ were released from the xenobiotic molecules by fungi selected from the microcosms.

Introduction

Marine environments contain a heterogenous microbial population in which the density of a given species varies with availability of nutrients and changes in the physical environment. Introduction of some xenobiotic molecules into the environment has a negligible effect on the microbial population, whereas other molecules may cause a drastic shift in microbial species and population numbers. The pollutants may eliminate the natural microbial population and interfere with the mineralization of nutrients.

To study the effects of microbial degradation of xenobiotic compounds, many investigators have used microcosms (Gordon et al., 1979; Pritchard et al., 1979). The microcosms are portions of the environment that have been removed from the natural environment and placed in a laboratory where the changes can be monitored and controlled.

The purpose of this study was to monitor the changes in the microfungal population of microcosms established from a salt water estuary which were challenged by carbaryl, methyl parathion and pentachlorophenol.

Materials and Methods

Microcosms. Two different microcosms were established, as described by Pritchard et al. (1979). One microcosm, a sediment-water test-system, was established from a core sample from the Range Point salt marsh (Pensacola Beach, Florida) consisted of 50 g of sand and detritis and 250 ml water (10–17 ppt. salinity) and was maintained at room temperature (23–25 °C) with the water-fraction aerated with a bubbler tube. The other microcosm, a continuous-flow system established from the same salt marsh, contained 144 g of sand, 250 ml detritis and 250 ml of water from the Range Point salt marsh layered into 1000 ml growth vessels. The temperature was maintained at 23 °C; salinity at 12–14 ppt. and air was provided at a rate of 30 cc/min.

Chemicals. Pentachlorophenol (UL ^{14}C, Pathfinder Lab., Inc.) was introduced into the sediment-water test system at 140 µg/l. Carbaryl (Naphthyl-l-^{14}C, California Bionuclear) and methyl parathion (2,6–^{14}C ring-labeled, Amerscham-Seral Corporation) were introduced into the continuous-flow microcosm from the reservoir at a rate of 14 ml/hr. to yield a final concentration of 214 µg/l in the growth vessel for the former and 75 µg/l for the latter.

Sampling method. At approximately weekly intervals, 1.0 ml samples of water and detritus were removed from

* ERL/GB Contribution No. 397.

0006-8055/80/0023-0129$02.00
© by Walter de Gruyter & Co. · Berlin · New York

NTIS is authorized to reproduce and sell this report. Permission for further reproduction must be obtained from the copyright owner.

each growth vessel. In addition, 1.0 ml samples of the headbox water, as well as detritus and water from the Range Point salt marsh, were examined for fungi. Each sample was vigorously mixed and inoculated onto mycological agar (Difco) by the spread plate method. The mycological agar contained 500 mg/l chloramphenicol to inhibit growth of bacteria. The plates were incubated for 1 week at (23–25 °C) and representative fungi were isolated and identified by conventional procedures.

Results

Prevalent fungi in the water samples from Range Point varied during the period of study, but *Trichoderma* was the most common fungus isolated from 10 of 13 sediment samples (Tab. I). A variety of fungi, in low numbers, were isolated from the headbox waters. Because of excessive rainfall in mid-July (> 40 cm rain), samples were not obtained from the Range Point salt marsh on July 12 and 23.

Addition of xenobiotics to the microcosms altered the pattern of species recovered (Tab. II). The prevalent fungus in the sediment of the carbaryl microcosm changed from *Trichoderma* to a *Fusarium* species. In the methyl parathion growth-vessel, *Trichoderma* predominated initially, but was succeeded by a *Penicillium* species. *Penicillium* I, provisionally classified within the *Penicillium chrysogenum* series, was isolated in large numbers in the final six samplings.

A different species, *Penicillium* II, provisionally classified within the *Penicillium canescens* series, predominated in the sediment of the pentachlorophenol sediment-water test-system during the sampling period.

Labeled xenobiotic molecules were added to batch-culture systems that contained only sterile seawater or dilute broth and fungi (Tab. III). The *Fusarium* sp. isolated from the carbaryl microcosm slowly released a small amount of $^{14}CO_2$ from the flask at approximately the same rate from both batch cultures, whereas *Trichoderma* sp. released negligible amounts of $^{14}CO_2$. Release of $^{14}CO_2$ from the carbaryl system in mycological broth ceased after 10 days and in seawater after 13 days. Only slight release of $^{14}CO_2$ from methyl parathion occurred in the *Penicillium* I batch culture. Larger amounts of $^{14}CO_2$ were released by *Penicillium* II in the pentachlorophenol broth-seawater cultures.

Tab. I. Prevalent fungi isolated from Range Point salt marsh during 1979

Date of Sample	Water	Sediment
March 12	*Epicoccum* (30)[1]	*Aspergillus* (70)
March 21	*Alternaria* (10)	*Trichoderma* (30)
March 29	*Cladosporium* (10)	*Trichoderma* (80)
April 5	*Cladosporium* (30)	*Trichoderma* (50)
April 11	*Penicillium* (40)	*Trichoderma* (TNC)
April 19	*Alternaria* (50)	*Cladosporium* (290)
April 26	*Cladosporium* (30)	*Trichoderma* (70)
June 8	(None)	*Cladosporium* (100)
June 14	*Trichoderma* (20)	*Trichoderma* (80)
June 22	*Trichoderma* (10)	*Trichoderma* (60)
June 27	*Penicillium* (10)	*Trichoderma* (80)
July 6	*Fusarium* (10)	*Trichoderma* (60)
July 26	*Aspergillus* (30)	*Trichoderma* (40)

[1]Genus of most commonly isolated fungus (number of fungi/ml)

Discussion

In previous studies of micro-ecosystems that contained labeled pesticides, more $^{14}CO_2$ was released from the sediment than from either the overlying water or the sand fraction (Pritchard et al. 1979), indicating that most of the degradation occurred in the sediment fraction. The role of fungi in these systems was not explored. *Trichoderma*, the most commonly isolated fungus from the sediment of the salt marsh, persisted as the most common fungus in sediments of the micro-ecosystems until xenobiotics were added.

Tab. II. Prevalent fungi in salt marsh microecosystem containing xenobiotic molecules

Pesticide	Environment	Prevalent Fungi Initially Isolated from Microecosystem			Prevalent Fungi Finally Isolated from Microecosystem		
		Fungus	Frequency	Range	Fungus	Frequency	Range
Carbaryl	Water	*Fusarium*[1]	3/7[2]	80–TNC[3]	*Fusarium*	6/8	30–340
	Sediment	*Trichoderma*	4/7	30–TNC	*Fusarium*	6/8	20–350
Methylparathion	Water	*Cladosporium*	3/7	20–80	*Penicillium* I	3/8	10–20
	Sediment	*Trichoderma*	6/7	60–TNC	*Penicillium* I	6/8	30–290
Pentachloro-phenol	Water	*Cladosporium*	3/7	20–120	*Penicillium* II	2/8	10–120
	Sediment	*Penicillium* II	5/7	40–950	*Penicillium* II	8/8	70–460

[1]Genus of most commonly isolated fungus.
[2]Number of times fungus isolated/number of weekly samples.
[3]Range of number of fungi isolated from 1 ml of sample.

Tab. III. Degradation of ^{14}C labelled xenobiotic molecules by fungi isolated from microecosystem

Fungus	Xenobiotic	Culture Medium	Days to Maximum $^{14}CO_2$ Evolution	Percent of Xenobiotic Molecules Degraded
Fusarium	Carbaryl	Dilute Mycological Broth	10	0.45
		Seawater	13	0.51
Trichoderma	Carbaryl	Dilute Mycological Broth	20	0.11
		Seawater	20	0.01
Penicillium I	Methyl-parathion	Dilute Mycological Broth	17	0.25
		Seawater	13	0.72
Penicillium II	Pentachlorophenol	Dilute Mycological Broth	20	0.17
		Seawater	20	1.74

A *Fusarium* species, selected from the carbaryl microcosm, in pure culture systems showed a capacity for release of $^{14}CO_2$ from labeled carbaryl. Such small amounts of $^{14}CO_2$ release suggest the degradation of a low concentration contaminant, but no contamination was detected by chemical analysis. Another possible reason for limited $^{14}CO_2$ release by the *Fusarium* sp. was that 1-naphthol was formed as a by-product; 1-naphthol is more toxic to fungi than is the parent carbaryl (Bollag, 1979). Similarly, selection for *Penicillium* II in the pentachlorophenol microecosystem was probably due to the inhibitory nature of this xenobiotic for many fungi.

These preliminary studies indicate that pollutant pesticides may select for fungi and possible alter normal recycling of nutrients in microhabitats Future studies will evaluate the use of microecosystems to predict rates of biodegradation by fungal activity and fate of select xenobiotic molecules.

Acknowledgements

We thank D. G. Ahearn for comments and assistance. This research was supported in part by grant no. R-80477 from the U.S. Environmental Protection Agency.

References

Bollag, J. M. 1979. Transformation of Xenobiotics by Microbial Activity. *In*: A. W. Bourquin and P. H. Pritchard, eds. *Proceedings of the Workshop: Microbial Degradation of Pollutants in Marine Environments.* EPA-600/9-79-012, Gulf Breeze, Florida. pp. 19–27.

Gordon, R. W., R. J. Beyers, E. P. Odum and R. G. Eagon. 1969. Studies of a simple laboratory microecosystem: bacterial activities in a heterotrophic succession. *Ecology 50*: 86–100.

Pritchard, P. H., A. W. Bourquin, H. L. Fredrickson and T. Maziarz. 1979. System design factors affecting environmental fate studies in microcosms. *In*: A. W. Bourquin and P. H. Pritchard, eds. *Proceedings of the Workshop: Microbial Degradation of Pollutants in Marine Environments.* EPA-600/9-79-012, Gulf Breeze, Florida pp. 251–272.

© Alle Rechte, insbesondere die der Übersetzung in fremde Sprachen, vorbehalten. Kein Teil dieser Zeitschrift darf ohne schriftliche Genehmigung des Verlages in irgendeiner Form — durch Photokopie, Mikrofilm oder irgendein anderes Verfahren — reproduziert oder in eine von Maschinen, insbesondere von Datenverarbeitungsmaschinen, verwendbare Sprache übertragen oder übersetzt werden.

All rights reserved, including those of translations into foreign languages. No part of this journal may be reproduced in any form — by photoprint, microfilm or any other means — nor transmitted nor translated into a machine language without written permission from the publisher.

NTIS is authorized to reproduce and sell this report. Permission for further reproduction must be obtained from the copyright owner.

600/J-80-098
Journal Article

Reprint from
BOTANICA MARINA
Walter de Gruyter · Berlin · New York

PB81-129512

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA 22161